## **CLAIMS**

- 1. A rat embryonic stem cell characterized by having the following properties (a)-(j):
- 5 (a) expressing Oct3/4 gene and Nanog gene,
  - (b) positive for alkaline phosphatase activity,
  - (c) having an embryoid body forming ability,
  - (d) expressing SSEA (Stage-Specific Embryonic Antigen)-1 and SSEA-4,
- (e) having the same number of chromosomes as does a normal rat cell,
  - (f) capable of being subcultured and holding the undifferentiated state,
  - (g) having in vitro pluripotency,
- (h) having a potential to differentiate for cells of three embryonic germ lineages,
  - (i) having teratoma formation ability,
  - (j) having an ability to produce a chimeric rat.
- 20 2. A rat embryonic stem cell obtained by performing a process comprising the following steps (A)-(D), under the culture conditions using a substantially serum free culture medium:
  - (A) a step for dissociating an inner cell mass formed by the culture of rat blastocysts, remaining a state of cell
- <sup>25</sup> aggregate,
  - (B) a step for culturing primary embryonic stem cells resulting from the culture of the dissociated inner cell mass until it can be passaged,
  - (C) a step for dissociating the primary embryonic stem cells,
- which have become capable of being passaged, remaining a state of cell aggregate, followed by passaging and culturing the same,
  - (D) a step for further passaging and culturing the cells to establish an embryonic stem cell.

- 3. The embryonic stem cell of claim 2, wherein the culture medium comprises a serum replacement reagent.
- The embryonic stem cell of claim 2 or 3, wherein the step
   (A) comprises a step for mechanically dissociating the inner cell mass.
- 5. The embryonic stem cell of any of claims 2 to 4, wherein the step (C) comprises a step for mechanically dissociating the embryonic stem cells.
  - 6. The embryonic stem cell of any of claims 2 to 5, wherein a culture medium without rat leukemia inhibitory factor (rLIF) is used in step (A).
  - 7. The embryonic stem cell of any of claims 2 to 6, wherein an rLIF-containing culture medium is used in steps (B)-(D).
- 8. A production method of a rat embryonic stem cell which comprises performing a process comprising the following steps (A)-(D), under the culture conditions using a substantially serum free culture medium:
- (A) a step for dissociating an inner cell mass formed by the culture of rat blastocysts, remaining a state of cell
   25 aggregate,
- (B) a step for culturing primary embryonic stem cells resulting from the culture of the dissociated inner cell mass

until it can be passaged,

- (C) a step for dissociating the primary embryonic stem cells, which have become capable of being passaged, remaining a state of cell aggregate, followed by passaging and culturing the same,
  - (D) a step for further passaging and culturing the cells to establish an embryonic stem cell.

- 9. The production method of claim 8, wherein the culture medium comprises a serum replacement reagent.
- 10. The production method of claim 8 or 9, wherein the step
  5 (A) comprises a step for mechanically dissociating the inner
  cell mass.
- 11. The production method of any of claims 8 to 10, wherein the step (C) comprises a step for mechanically dissociating the embryonic stem cells.
  - 12. The production method of any of claims 8 to 11, wherein an rLIF-free culture medium is used in step (A).
- 15 13. The production method of any of claims 8 to 12, wherein an rLIF-containing culture medium is used in steps (B)-(D).
- 14. A subculture method of rat embryonic stem cells which comprises dissociating and passaging the cells, remaining a 20 state of cell aggregate.
  - 15. The subculture method of claim 14, which comprises a step for mechanically dissociating the cells.
- 25 16. The subculture method of claim 14 or 15, wherein the cells are cultured using a substantially serum free culture medium.
  - 17. The subculture method of claim 16, wherein the culture medium comprises a serum replacement reagent.
  - 18. The subculture method of claim 16 or 17, wherein the culture medium comprises rLIF.
- 19. A culture medium for rat embryonic stem cell, which comprises a serum replacement reagent and rLIF.

- 20. A culture kit for rat embryonic stem cell, which comprises a serum replacement reagent and rLIF as components.
- 5 21. The culture kit of claim 31, which further comprises the rat embryonic stem cell of any of claims 1 to 7 as a component.
  - 22. The culture kit of claim 20 or 21, which further comprises feeder cells as a component.
  - 23. The culture kit of claim 22, wherein the feeder cells are embryo-derived normal fibroblasts.

- 24. A differentiation induction method of a rat embryonic stem cell, which comprises stimulating the rat embryonic stem cell of any of claims 1 to 7 with a differentiation inducer.
- 25. The differentiation induction method of claim 24, wherein the differentiation inducer is a retinoic acid, growth factor, glucocorticoid or extracellular substrate.
  - 26. A cell obtained by inducing the differentiation of the rat embryonic stem cell of any of claims 1 to 7.
- 25 27. A cDNA library, genomic library or cell extract derived from the rat embryonic stem cell of any of claims 1 to 7.
  - 28. A screening method of a differentiation inducer for tissue or cell, which comprises the following steps (i)-(iii):
- (i) a step for bringing a test substance into contact with the rat embryonic stem cell of any of claims 1 to 7,
  - (ii) a step for evaluating the presence or absence or the extent of the differentiation of the rat embryonic stem cell,
  - (iii) a step for judging whether or not the test substance is
- $^{35}$  a substance associated with differentiation induction, based

on the evaluation results of the above-mentioned (ii).

- 29. A screening method of a substance acting on the differentiation induction of tissue or cell, which comprises the following steps (I)-(III):
  - (I) a step for bringing a test substance into contact with the rat embryonic stem cell of any of claims 1 to 7,
  - (II) a step for culturing the rat embryonic stem cell of the aforementioned (I) under the conditions allowing
- differentiation induction of the embryonic stem cell, and evaluating the presence or absence or the extent of the differentiation thereof,
- (III) a step for judging whether or not the test substance is a substance acting on the differentiation induction of tissue or cell, based on the evaluation results of the abovementioned (II).
  - 30. A use of the rat embryonic stem cell of any of claims 1 to 7 in the production of a genetically modified rat.
  - 31. The use of claim 30, wherein the genetically modified rat is either of a chimeric rat, knockout rat, knockin rat, transgenic rat and knockdown rat.
- 25 32. A production method of a genetically modified rat, which comprises performing a process comprising the following steps (X)-(Z):
  - (X) a step for introducing a desired gene into the rat embryonic stem cell of any of claims 1 to 7,
- (Y) a step for preparing an oocyte for transplantation comprising the rat embryonic stem cell into which the gene was introduced,
  - (Z) a step for transferring the oocyte for transplantation into a pseudopregnant female rat to produce an offspring rat.

- 33. A genetically modified rat produced by the production method of claim 32.
- 34. The rat of claim 33, which is either of a chimeric rat, knockout rat, knockin rat, transgenic rat and knockdown rat.